

*Supplementary files for:*

**Two-stage electro-mechanical coupling of a Kv channel in voltage-dependent activation**

Panpan Hou, Po Wei Kang, Audrey Deyawe Kongmeneck, Nien-Du Yang,  
Yongfeng Liu, Jingyi Shi, Xianjin Xu, Kelli McFarland White, Mark A. Zaydman, Marina A.  
Kasimova, Guiscard Seebohm, Ling Zhong, Xiaoqin Zou, Mounir Tarek\*, and Jianmin Cui\*

\*To whom correspondence should be addressed. E-mail: [jcui@wustl.edu](mailto:jcui@wustl.edu) (J.C.) or  
[mounir.tarek@univ-lorraine.fr](mailto:mounir.tarek@univ-lorraine.fr) (M.T.)

**This PDF File Includes:**

Supplementary Table 1

Supplementary Figures. 1 to 7

**Supplementary Table 1. All primer sequences used in this study.**

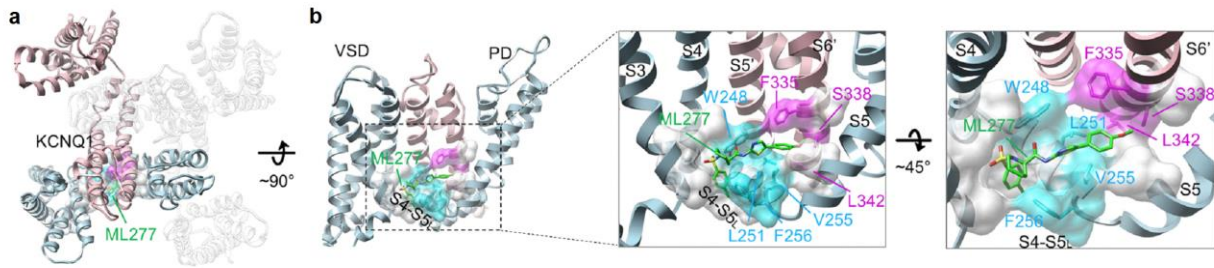
<b>Mutations</b>	<b>Primer sequences</b>
<b>C214A</b>	pB: cttggaTcccacCGCgaggaccacca pC: cGCGgtgggAtccaaggggcaggtg
<b>G219C</b>	pB: cctgaCacttgaTcccacCGC pC: ggAtccaagtGtcaggtgttgccacg
<b>C331A</b>	pB: gacagagaaTGCggaggcgatggtcttc pC: ctccGCAttctctgtctttgccatc
<b>E160R</b>	pB: cgatTCTcatccagaagagagt pC: ctctctggatgAGAatcgtgctggtggtgttct
<b>R231E</b>	pB: caggaaTTCgatgccctgatggc pC: gggcatcGAAttctgcagatcctgag
<b>R237E</b>	pB: gcatcTCcaggatctgcaggaag pC: cagatcctgGAgatgctacacgtcgac
<b>F127A</b>	pB: gaagacggcgGCAtggtaaacgaagcatttc pC: caTGCcgccgtcttctcatc
<b>K183N</b>	pB: cacgtaAttgctgcggcagcc pC: ccgcagcaaTtacgtgggcctctgg
<b>R190Q</b>	pB: gcagcTgccccagaggcc pC: ctgggggcAgctgcgctttgccc
<b>F193A</b>	pB: cgTgcaGCgcgcagccgcccc pC: ctgcgcGCtgcAcggaagcccatttcc
<b>R195A</b>	pB: ggcttAGCggcaaagcgcagccg pC: gctttgccGCTaagcccatttccatcatc
<b>K196E</b>	pB: ctgcgctttgcccgGagcccatttc pC: ccggGagcccatttccatcatc
<b>P197A</b>	pB: ggaaatTGCcttcgggcaaagcg pC: ccggaagGCAatttccatcatcgacctc
<b>R237W</b>	pB: gcatccAcagTatctgcaggaagcgg pC: cagatActgTggatgctacacgtcg
<b>Q244W</b>	pB: cctccccaccggtcgacgtgtagc pC: gaccggtggggaggcacctggag
<b>G245W</b>	pB: ccagggtccccactggcggtcgacgtg pC: cagtgggggacctggaggctcct
<b>G246W</b>	pB: ccagggtccaaccctggcggtcgac pC: cagggttgacctggaggctcctg
<b>T247W</b>	pB: gagtctccaccagcctccctggcggtc pC: ggctggtggagactcctgggctccgtg
<b>W248R</b>	pB: cctccGggtgctccctggc pC: gaggcaccCggaggctcctgggc
<b>R249W</b>	pB: gagccAccaCgtgctccttggcg pC: ggcaCgtgTggtcctgggctcc
<b>L250W</b>	pB: gagccGagCCAacctcaggtgcctc pC: gaggTGGctCggctccgtgtcttc

<b>L251A</b>	pB: cggaTcccGCgagcctccaggtgcc pC: gctcGCgggAtccgtggtcttcatcc
<b>G252W</b>	pB: gaccacTgaCcAcaggagcctccaggtg pC: cctgTgGtcAgtggtcttcatccaccg
<b>S253W</b>	pB: gaccacCCagcccaggagcctcc pC: gggctGGgtggtcttcatccacc
<b>V254M</b>	pB: gaagaccaTggagcccaggagcc pC: ggctccAtggtcttcatccaccg
<b>V255W</b>	pB: gatgaaCCAacaggagcccaggag pC: ctccgtgTGGttcatccaccgccagg
<b>F256A</b>	pB: gtggatgGCgaccacggagcccag pC: gtggtcGCcatccaccgccagg
<b>I257W</b>	pB: gcggtgCCAAaagaccacggagccc pC: ggtcttTTGGcaccgccaggagctg
<b>H258W</b>	pB: ctggcgCCAgatgaagaccacggag pC: cttcatcTGGcgccaggagctgataac
<b>R259W</b>	pB: cagctcctgCcAgtggatgaagaccacgg pC: cacTgGcaggagctgataaccac
<b>Q260W</b>	pB: gctccCACcggtggatgaagaccac pC: ccaccgGTGggagctgataaccacc
<b>E261K</b>	pB: gttatAagctTctggcggtggatgaag pC: cgccagAagctTataaccaccctgtacatc
<b>L262A</b>	pB: ggtggtAatTGCctcctggcggtggatg pC: ggagGCAatTaccaccctgtacatcg
<b>I263A</b>	pB: ggtggttGCcagctcctggcggtg pC: gagctgGCaaccaccctgtacatc
<b>T264A</b>	pB: cagTgtggCtatcagctcctggcg pC: ctgataGccacActgtacatcg
<b>T265A</b>	pB: gtacagAgCggttatcagctcctg pC: gataaccGcTctgtacatcggttc
<b>L266A</b>	pB: GATAACCACCGCCTACATCG pC: CGATGTAGGCGGTGGTTATC
<b>Y267A</b>	pB: ccgatAGCcagggtggttatcagc pC: caccctgGCTatcggttctctgggcc
<b>I268W</b>	pB: gaagccCCAgtacagggtggttatcag pC: cctgtacTGGggttctctgggcctc
<b>G269A</b>	pB: CTGTACATCGCCTTCCTGGGCCT pC: AGGCCCAGGAAGGCGATGTACAG
<b>F270A</b>	pB: GTACATCGGCGCCCTGGGCCTC pC: GAGGCCAGGGCGCCGATGTAC
<b>L271E</b>	pB: gatgagggcTTCgaagccgatgtacagg pC: ctteGAAggectcatettctcc
<b>G272A</b>	pB: CTTCTGGCCCTCATCTTCT pC: AGAAGATGAGGGCCAGGAAG
<b>L273F</b>	pB: gaagatgaATccaggaagccgatg pC: cctgggATtcatcttctctctgac

<b>I274A</b>	pB: TGGGCCTCGCCTTCTCGAGCTA pC: TAGCTCGAGAAGGCGAGGCCCA
<b>F275A</b>	pB: GCCTCATCGCCTCGAGCTA pC: TAGCTCGAGGCGATGAGGC
<b>F335A</b>	pB: CTTCTCTGTCTGCTGCCATCTCC pC: CTTCTCTGTCTGCTGCCATCTCC
<b>A336G</b>	pB: gaaggaAatTCcaaagacagagaagcag pC: gtctttgGAatTtccttcttgcgctcc
<b>I337A</b>	pB: CTGTCTTTGCCGCCTCCTTCTTTGC pC: GCAAAGAAGGAGGCGGCAAAGACAG
<b>S338F</b>	pB: gaagAATatTgcaaagacagagaag pC: gtctttgcAatATTcttcttgcgctccc
<b>F339S</b>	pB: gcaaaACTggagatggcaaagacag pC: ccattctccAGTtttgcgctcccagcg
<b>F340A</b>	pB: GCCATCTCCTTCGCTGCGCTCCCA pC: TGGGAGCGCAGCGAAGGAGATGGC
<b>A341V</b>	pB: gggagcAcaaagaaggagatggc pC: ccttctttgTgctcccagcgggg
<b>L342A</b>	pB: CTTCTTTGCGGCCCCAGCG pC: CGCTGGGGCCGCAAAGAAG
<b>P343A</b>	pB: ccgcGgCgagcgcaaagaaggag pC: gcgctcGcCgcggggattcttggc
<b>A344V</b>	pB: gaatccccActgggagcgcaaagaag pC: ctcccagTggggattcttggctcg
<b>G345A</b>	pB: gccaagaateGccgctgggagcgc pC: gcggCgattcttggctcggg
<b>I346A</b>	pB: gagccaagGGCccccgctgggagcgc pC: gggGCCcttggctcggggtttg
<b>L347A</b>	pB: gagccaGCaatccccgctgggagc pC: ggggattGctggctcggggtttgcc
<b>G348A</b>	pB: ccgaCGcaagaatccccgctg pC: ggattcttgCGtcgggggtttgccctg
<b>S349A</b>	pB: gcaaaccgCgccaagaatccccgc pC: ggcGcggggtttgccctg
<b>G350A</b>	pB: ggcaaacGccgagccaagaatcc pC: gctcggCgtttgccctgaagggtg
<b>F351A</b>	pB: ggcaGCGcccagccaagaatcc pC: gctcgggCGCtgccctgaagggtcag
<b>A352C</b>	pB: GATCCGGGTTTTGCCTGAAGGTGCAG pC: CTGCACCTTCAGGCAAACCCGGATC
<b>L353K</b>	pB: ccttcTTggcaaaccgagc pC: gggtttgccAAgaaggtgcagcagaag
<b>K354A</b>	pB: ctgcacGGCagggcaaaccg pC: ccctgGCCgtgcagcagaagcag
<b>V355W</b>	pB: gggtttgccctgaagTGgcagc pC: gggtttgccctgaagTGgcagc

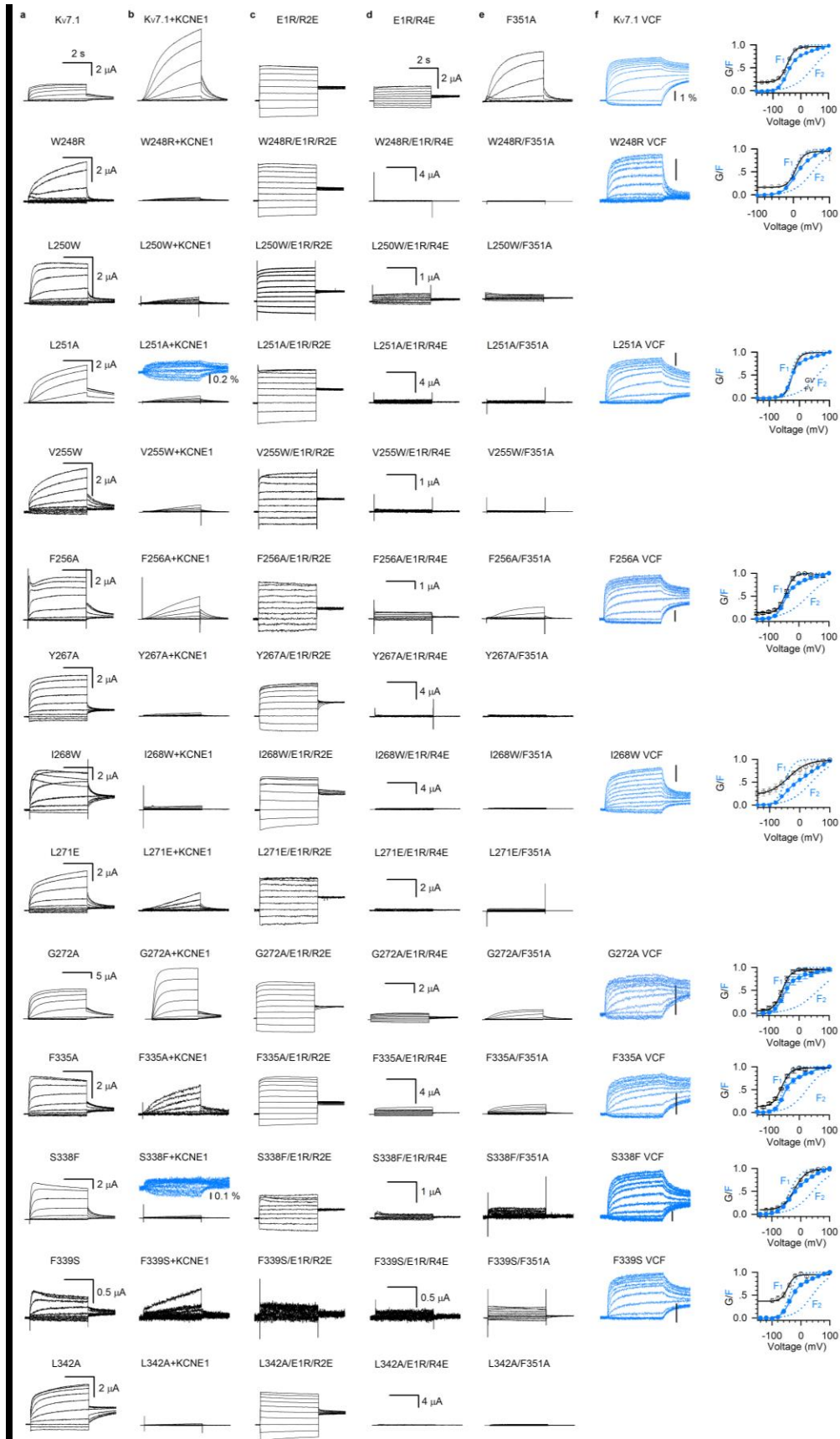
<b>Q356A</b>	pB: GTTTGCCCTGAAGGTGGCGCAGAAGCAG pC: CTGCTTCTGCGCCACCTTCAGGGCAAAC
<b>Q357A</b>	pB: GAAGGTGCAGGCGAAGCAGAGGCAGA pC: TCTGCCTCTGCTTCGCCTGCACCTTC
<b>K358A</b>	pB: GTGCAGCAGGCGCAGAGGCAGAAGCAC pC: GTGCTTCTGCCTCTGCGCCTGCTGCAC
<b>Q359A</b>	pB: GTGCAGCAGAAGGCGAGGCAGAAGCAC pC: GTGCTTCTGCCTCGCCTTCTGCTGCAC
<b>R360A</b>	pB: CAGCAGAAGCAGGCGCAGAAGCACTTC pC: GAAGTGCTTCTGCGCCTGCTTCTGCTG
<b>M238W</b>	pB: gacgtgCagcCAcctcaggatctgcagg pC: gaggTGgctGcacgtcgaccgccag
<b>E160R/ R237E/ M238W</b>	Use E160R/R237E as template M238W-pB: gacgtgCagcCAcTCcaggatctgcagg M238W-pC: GAgTGgctGcacgtcgaccgccag
<b>L239W</b>	pB: cgacgtgCCAcatcctcaggatctgc pC: ggatgTGGcacgtcgaccgccag
<b>E160R/ R237E/ L239W</b>	Use E160R/R237E as template L239W-pB: gacgtgCCAcatcTCcaggatctgc L239W-pC: GAgatgTGGcacgtcgaccgccag
<b>D242W</b>	pB: gcgCCAgacgtgtagcatctc pC: ctacacgtcTGGcgccagggaggcac
<b>R243W</b>	pB: ccctgCcAgtcgacgtgtagcatc pC: gtcgacTgGcagggaggcacctgg
<b>I268A</b>	pB: caggaaTccgGCgtacaggggtggtatcag pC: gtacGCcggAttcctgggcctcatcttc
<b>L251F</b>	pB: gagccGaAgagcctccagggtgcc pC: gaggctcTtCggetccgtggtcttc
<b>F339L</b>	pB: cgcGaaGaGggagatggcaaagacag pC: catctccCtCttCgcgtcccageggg

## Supplementary Figures



### Supplementary Fig. 1, Molecular docking results of ML277 onto KCNQ1 channel.

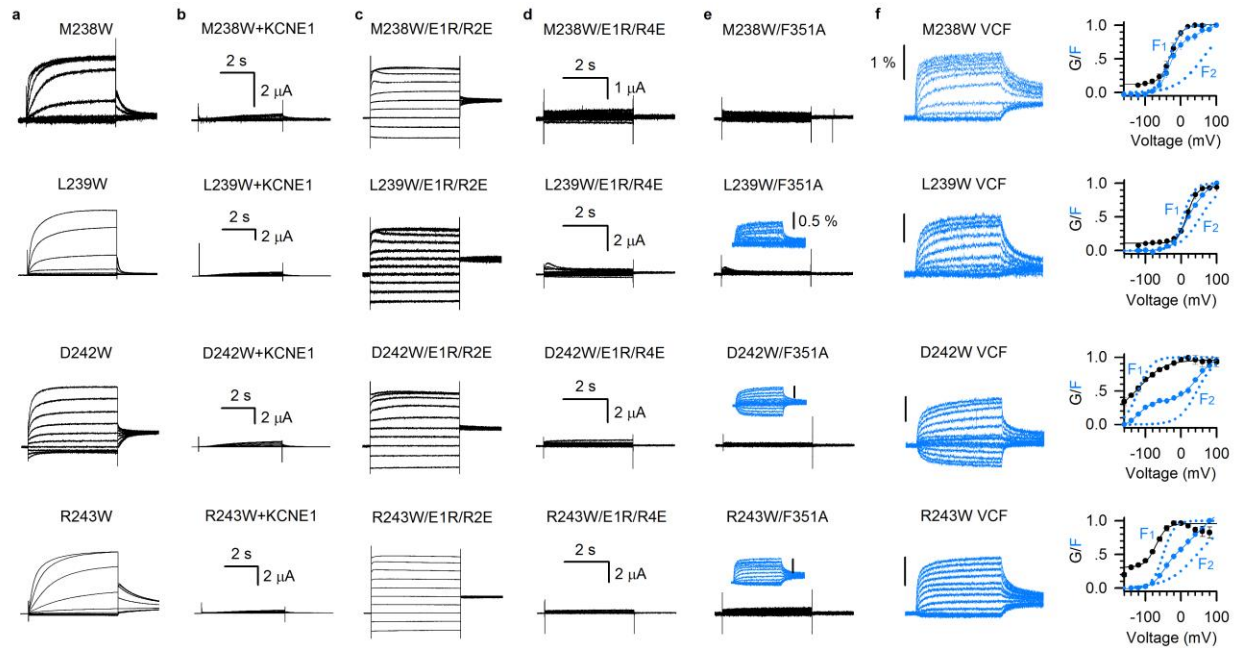
**(a)** Residues interacting with ML277 based on molecular docking and experimental results. Top view of the binding mode of ML277 (green) at the homology model of hKv7.1 (see Methods). Two neighboring subunits (light red and light blue) are highlighted. **(b)** Side view of the binding mode of ML277. Only two neighboring subunits (light red and light blue) are shown for clarity. Right panels, detailed structure of the ML277 (green) binding at the S4-S5L/pore interface. Interacting residues that consistent with mutagenesis results shown in cyan (S4-S5L) and magenta (pore).



**Supplementary Fig. 2. Dataset for functional validations of AO state E-M coupling residues identified by the ML277 screen.**

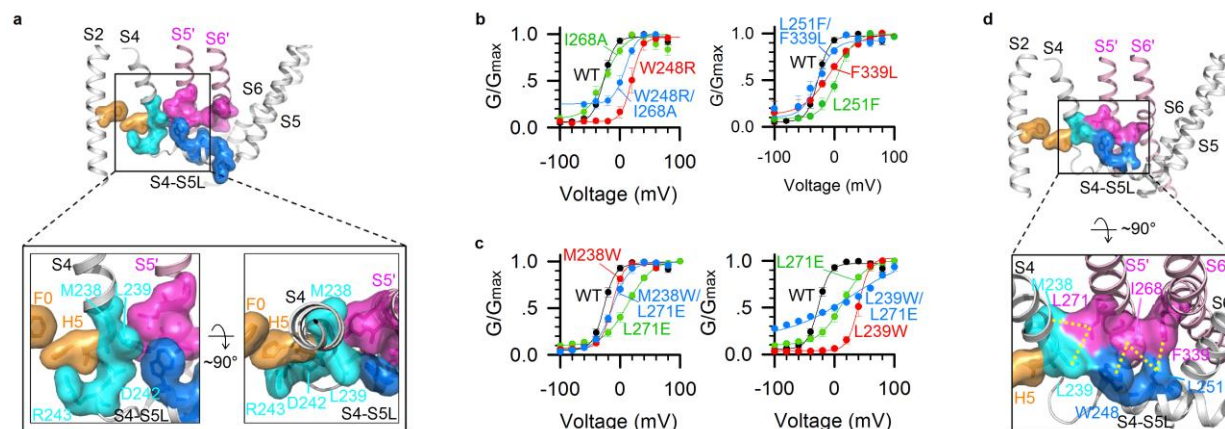
**(a-b)** Currents of WT and mutant K<sub>v</sub>7.1 with and without KCNE1. Currents are shown in the same scale. VCF experiments was performed on S338F+KCNE1 and L251A+KCNE1, indicating membrane expression and VSD function. **(c-e)** Currents of WT and mutant K<sub>v</sub>7.1 on top of the IO background E1R/R2E, the AO background E1R/R4E and F351A. Currents are shown in the same scale. **(f)** VCF results of WT and eight mutations in K<sub>v</sub>7.1\*.  $n \geq 3$ . Source data are provided as a Source Data file.





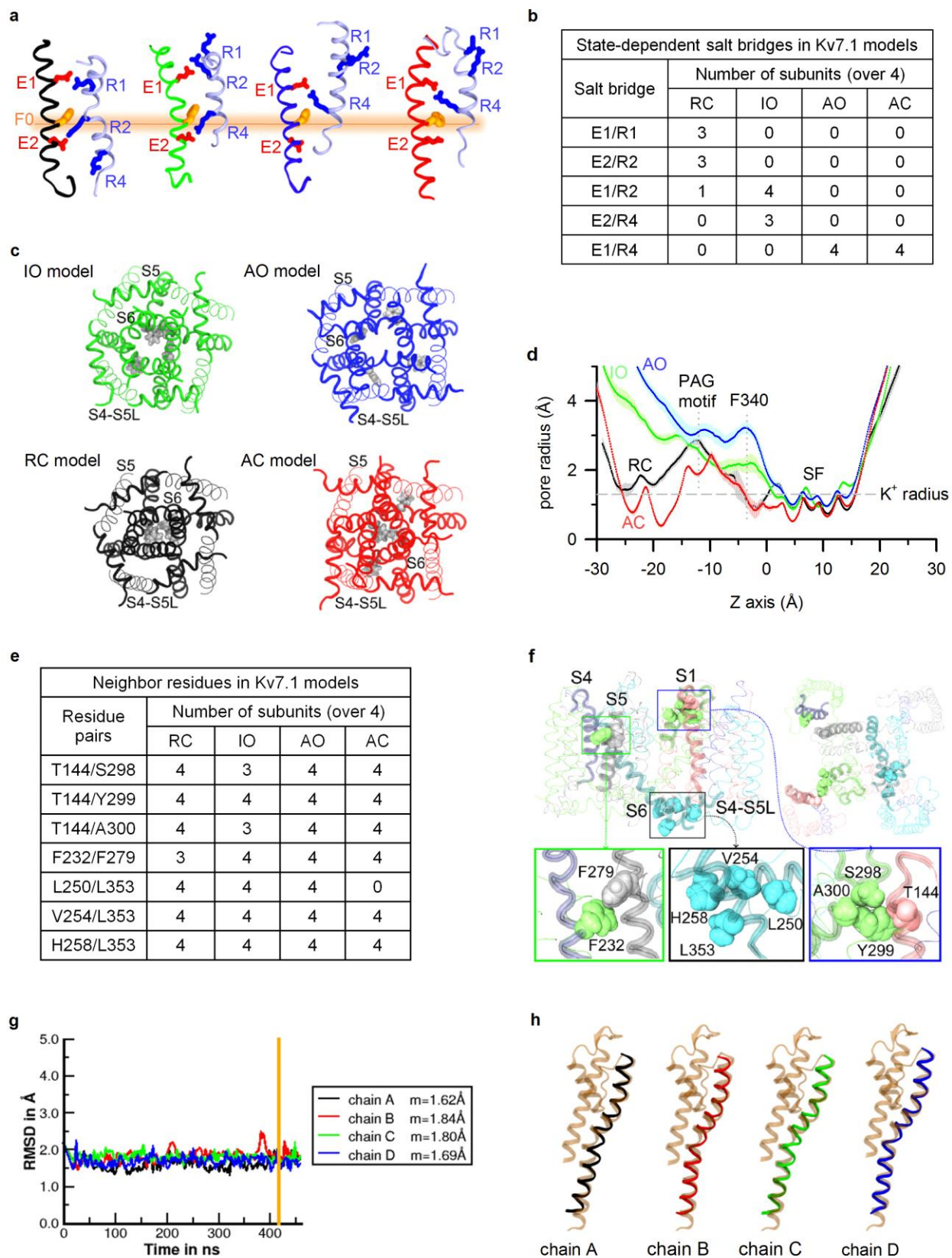
**Supplementary Fig. 3. Dataset for functional validations of S4c residues critical for AO state E-M coupling.**

(a-b) Currents of S4c mutations with and without KCNE1. Currents are shown in the same scale. (c-e) Currents of the S4c mutations on top of the IO background E1R/R2E, the AO background E1R/R4E and F351A. Currents are shown in the same scale. VCF results (three out of four) are shown in blue. (f) VCF results of the four mutations in S4c in  $K_v7.1^*$ .  $n \geq 3$ . Source data are provided as a Source Data file.



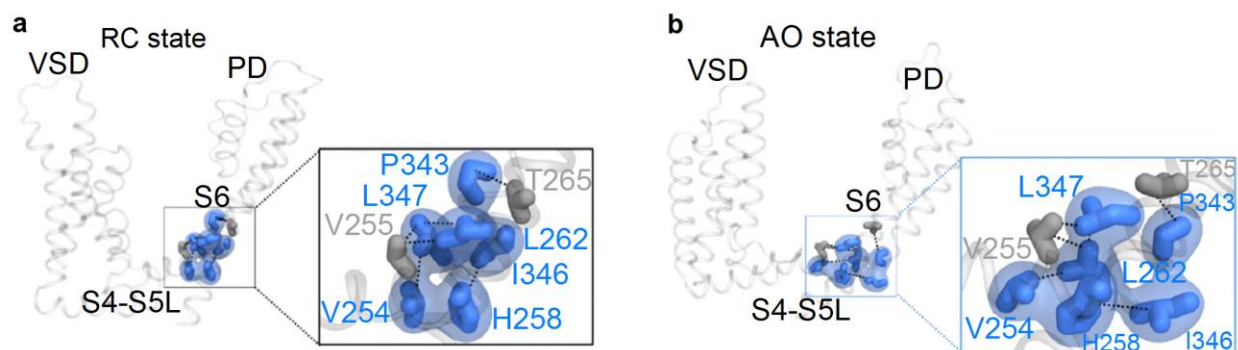
**Supplementary Fig. 4. Double mutant cycle analysis.**

(a) Mapping the key residues on to the Kv7.1 cryoEM structure, the VSD of which is at the activated state. Cyan, S4c residues (M238, L239, D242, and R243); Orange, F0 and H5 in the charge transfer center; Blue, S4-S5L residues; Pink, pore residues. Lower panels, detailed structure of the key residues. (b-c) Double mutant cycle analysis of the interactions at S4-S5L/pore interface: W248 and I268 ( $\Delta\Delta G = 1.4$  kcal/mol) and L251 and F339 ( $\Delta\Delta G = 2.7$  kcal/mol), as well as the S4c/S5 interface: M238 and L271 ( $\Delta\Delta G = 1.7$  kcal/mol) and L239 and L271 ( $\Delta\Delta G = 4.4$  kcal/mol).  $G$ - $V$  relations from WT and mutations are fitted with Boltzmann equation.  $n \geq 3$ . (d) Mapping the interactions M238/L271 and L239/L271 at the S4c/S5 interface, and W248/I268, L251/I268, and L251/F339 at the S4-S5L/pore interface. Color code is the same as in a, and yellow dashed lines indicate the interactions.



**Supplementary Fig. 5. Structural validation of the MD trajectories obtained for Kv7.1 models.**

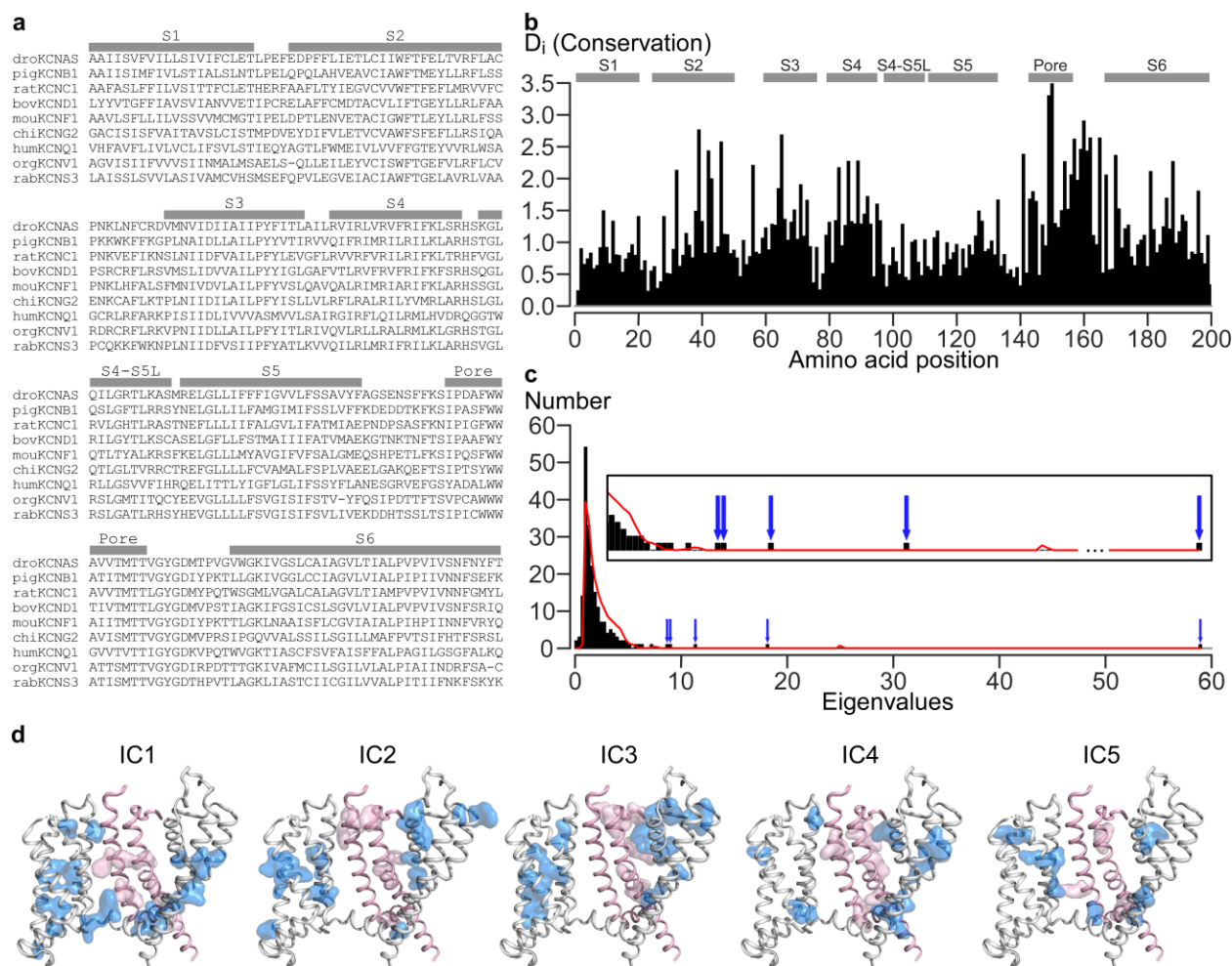
**(a)** State-dependent Kv7.1 VSD salt-bridges<sup>1-4</sup>. Representation of S2 (in black, green, blue and red) and S4 (in blue) segments in Kv7.1 RC, IO, AO and AC models, respectively. S4 gating charges are represented in blue sticks, while its S2 countercharges are represented in red sticks. The hydrophobic plug F167 (F0) is represented in orange sticks. **(b)** Summary table of state-dependent Kv7.1 VSD salt-bridges in the subunits of Kv7.1 models. **(c)** Intracellular view of S4-S5L and PD segments in Kv7.1 IO (top, left), AO (top, right), RC (bottom, left) and AC (bottom, right) models. The gray spheres are sidechains of F340. **(d)** Average pore radii of the conduction pathways of Kv7.1 models. Standard deviations are represented by black, green, blue and red horizontal bars for the MD trajectories obtained from RC, IO, AO and AC models, respectively. **(e)** Summary table of state-dependent KCNQ1 neighbor residues pairs identified by cysteine scanning studies and previous double mutant cycle analysis in the subunits of KCNQ1 RC, IO, AO and AC models involving T144<sup>5</sup>, F232<sup>6</sup>, and L353<sup>7</sup>. In this analysis we calculated the distance between C $\beta$  atoms of the residue pairs with a cut-off of 13 Å to determine if these residues are prone to interact when mutated into Cys. **(f)** Interacting pairs of KCNQ1 neighbor residues. In the upper panel, the side view (left) and the extracellular view (right) of the seven pairs of neighbor residues in the KCNQ1 IO model are shown. The transmembrane segments (in cartoon) bearing the residues of interest are highlighted in transparent colors. In the lower panel, the zoomed views of IO state specific interaction in IO model (left), RC state specific interactions in RC model (middle), and of AO state specific residue pairs in AO model (right) are displayed. **(g-h)** Comparison of the pore domain of Kv7.1 RC model with KCNQ1<sub>EM</sub> structure. **(g)** The graph project the root-mean-square deviation (RMSD) of the pore domain segments of Kv7.1 RC model with respect to KCNQ1<sub>EM</sub> structure throughout its MD simulation time (in ns). Average RMSD values are displayed for each subunit (labeled chain A, B, C, D). The orange vertical line represents the simulation time at which the pictures displayed in (h). **(h)** A cartoon representation of the structural alignment of the S6 segment of KCNQ1<sub>EM</sub> structure (in transparent orange) with those of Kv7.1 (top) RC subunits, each captured at ~420 ns. Each subunit is represented in solid black, red, green and blue cartoon, respectively. Source data are provided in Source Data file.



**Supplementary Fig. 6. Possible intra-subunit interactions at the classical E-M coupling region in RC and AO states.**

**(a-b)** Representations of the IO and AO state models of a Kv7.1 subunit. Inset: enlarged structures of the boxed area. Blue, residues identified as important for E-M coupling in experiments (Fig. 1c-g); grey: V255 and T265, which were identified in MD simulations as important for E-M coupling, but for which mutations still show functional currents (Fig. 3c). Residue V255 was found to be important for the AO state E-M coupling (Fig. 3). Each pair of residues is represented by a black dashed line connecting their respective sidechains.





**Supplementary Fig. 7. Statistical coupling analysis of domain-swapped Kv channels.**

(a) Expanded sample MSA (from Figure 6a) used as input for analysis by SCA. MSA uses the nomenclature established by the HUGO Gene Nomenclature Committee (HGNC) for voltage-gated potassium channels<sup>8</sup>. Kv channels can adopt two types of architectures. In “domain-swapped” channels, the VSD from one subunit contacts the PD from the neighboring subunit. In “non-domain-swapped” channels, the VSD from one subunit contacts the PD from the same subunit. These distinct architectures likely give rise to different modes of E-M coupling. SCA was applied to the Kv channel superfamily previously<sup>9</sup>; however, prior studies utilized both domain-swapped and non-domain swapped Kv channels in the input data. In this MSA, only domain-swapped Kv channels were included. One member from each of Kv1 – Kv9 is shown. The final processed MSA includes 1421 sequences at 200 positions. (b) First order conservation by amino acid position within the MSA.  $D_i$  on the y-axis is calculated based on Kullback-Leibler divergence which measures deviation of the observed amino acid frequency in the MSA (Fig. 6a)

from the natural “background” amino acid frequency (see Methods). Bars indicate positions of the helical segments. **(c)** A histogram of eigenmodes tallied by their associated eigenvalues from SCA calculation (black). Red indicates the spectrum calculated by randomizing the input. Five significant eigenmodes can be seen by the black bars rising above the randomized red curve (blue arrows) at high eigenvalues. Inset shows enlarged scales. **(d)** Amino acid residues within independent component 1 through independent component 5 identified by SCA mapped onto the Kv7.1 cryoEM structure. Blue surfaces indicate all IC positions on one subunit, pink surfaces indicate the same IC positions on the neighboring subunit. Only the S5 and S6 helices are shown on the neighboring subunit for sake of clarity (pink helices). Accordingly, only the IC positions on the neighboring subunit on S5 and S6 are shown (pink surfaces). Using the IC submatrix and based on the strength of external correlation (see Methods, Figure 6c), IC2+IC3 are combined to form sector 1 (Fig. 6d), while IC1+IC4+IC5 are combined to form sector 2 (Fig. 6e). Source data for SCA are provided in Source Data file.

## References

1. Hou, P. et al. Inactivation of KCNQ1 potassium channels reveals dynamic coupling between voltage sensing and pore opening. *Nat Commun* **8**, 1730 (2017).
2. Hou, P., Shi, J., White, K. M., Gao, Y. & Cui, J. ML277 specifically enhances the fully activated open state of KCNQ1 by modulating VSD-pore coupling. *Elife* **8** (2019).
3. Zaydman, M. A. et al. Domain-domain interactions determine the gating, permeation, pharmacology, and subunit modulation of the IKs ion channel. *Elife* **3**, e03606 (2014).
4. Wu, D., Pan, H., Delaloye, K. & Cui, J. KCNE1 remodels the voltage sensor of Kv7.1 to modulate channel function. *Biophys J* **99**, 3599-608 (2010).
5. Xu, Y. et al. Building KCNQ1/KCNE1 channel models and probing their interactions by molecular-dynamics simulations. *Biophys J* **105**, 2461-73 (2013).
6. Nakajo, K. & Kubo, Y. Steric hindrance between S4 and S5 of the KCNQ1/KCNE1 channel hampers pore opening. *Nat Commun* **5**, 4100 (2014).
7. Choveau, F. S. et al. KCNQ1 channels voltage dependence through a voltage-dependent binding of the S4-S5 linker to the pore domain. *J Biol Chem* **286**, 707-16 (2011).
8. Gutman, G. A. et al. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* **57**, 473-508 (2005).
9. Lee, S. Y., Banerjee, A. & MacKinnon, R. Two separate interfaces between the voltage sensor and pore are required for the function of voltage-dependent K(+) channels. *PLoS Biol* **7**, e47 (2009).